Antibiotic risk assessment needs to protect both environmental and human health

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In our recent meta-analysis on antibiotic ecotoxicity data published in *Environment International* (Le Page et al. 2017) we suggest that because of the great diversity in species sensitivity, environmental risk assessment (ERA) would be improved by testing a more diverse range of bacteria (including both environmental bacteria and clinically relevant bacteria (CRB)). We also conclude that tests on antibiotics should consider endpoints of relevance to ecosystem function. Comparing the protection goals for environmental heath with those for human health (protection against antimicrobial resistance (AMR) development) we, furthermore, identify that neither protection goal is always protective of the other whilst using current methodologies (with surrogate endpoints for each goal and very limited bacterial biodiversity tested); supporting the need for both in any comprehensive health protection system for antibiotics.

In a correspondence to our paper Bengtsson-Palme and Larsson (2018) point out a bias in our sensitivity analysis favouring environmental bacteria (including cyanobacteria). We acknowledge this, but equally in this correspondence we challenge some of their points made on how this impacts on the significance of

our data. We also address points relating to the lack of clarity on protection goals for antibiotics in the discussion of our paper and discuss what data are most suitable for establishing those protection goals. We emphasise that the main conclusion drawn from our original paper has not changed and we maintain that a holistic approach including both environmental health and resistance selection is required to drive an effective overall protection limit for antibiotics.

Sensitivity analyses skews

Bengtsson-Palme and Larsson (2018) rightfully point out that our analysis skews the apparent sensitivity in favour of the environmental bacteria because the endpoints compared for CRB (minimum inhibitory concentrations, MIC) and environmental bacteria (no observed effect concentrations, NOEC) for growth inhibition are derived from different ends of the dose response curve; MICs are derived from the top of the dose-response curve (full inhibitory effect on growth) and the NOECs for environmental bacteria from the bottom of the response curve (concentration with no inhibition). In some cases therefore CRB may be more sensitive than environmental bacteria than our analysis suggests. However, it should be highlighted that this doesn't necessarily mean that environmental bacteria will not represent the most sensitive taxa for individual antibiotics. This is because, in the first instance, in the cases where environmental bacteria were more sensitive by an order of magnitude or more compared with CRB in our analysis, environmental bacteria are likely to be comparable, if not more sensitive to those antibiotics. In our meta analysis this would be the case for 6 out of 24 antibiotics (including azithromycin and ampicillin). Secondly, very large differences in sensitivity can occur between different species of bacteria (our meta analysis showed sensitivity spanned five orders of magnitude in 8 species cyanobacteria exposed to ampicillin) and because of the far greater species number and diversity tested in CRB compared with environmental bacteria there is likely to be a sensitivity bias in favour of CRB. The size-adjusted MIC value used as our comparative endpoint for CRB was calculated from the MICs of up to 70 species in up to 5 families (Bengtsson-Palme and Larsson 2016). In stark contrast to CRB, cyanobacteria antibiotic test data

were generally derived from only one or two species giving far greater uncertainty in the sensitivity calculation for this group.

Uncertainty in protection targets.

ERA for antibiotics in the European Union is legislated by the Medicinal Products for Human Use directive (EC 2001) where the protection goal is to prevent "any risk of undesirable effects on the environment". Current practice is to calculate a PNEC using chronic growth and/or reproduction data on single species, which for antibiotics is normally based on the $PNEC_{SW}$ driven by a cyanobacterium. The relationship however, between individual species sensitivity, ecosystem function and functional redundancy is not well understood (Antwis et al. 2017) and what constitutes an "undesirable effect" is unclear. As Bengtsson-Palme and Larsson (2018) point out, clarity is, therefore, required in the definition and objectives of these protection goals. The issue of functional redundancy, and to what extent it is possible to eradicate or lose a microbial species without compromising that ecosystem function is a hugely important consideration for environmental protection. There is some evidence that microbial communities may be less functionally redundant than macroorganism communities (Delgado-Baquerizo et al. 2016). Thus, although we re-iterate our support of the inclusion of ecosystem function based tests, given the uncertainties relating to functional redundancy, at this time ecosystem level protection may be best served by a conservative protection goal based upon bacterial biodiversity (and therefore inherently ecosystem function).

Bengtsson-Palme and Larsson (2018), highlight that the risk of AMR and human health concerns are generally the main driving force for antibiotic protection goals but they also agree with our conclusions that a holistic approach that considers both environmental health and AMR should be taken. The meta analysis shows that for some antibiotics the environmental protection limits may be lower than the protection limits predicted for AMR (using current methodologies and surrogate endpoints for biodiversity and AMR). To illustrate this, here (Fig 1) we compare the $PNEC_r$ determined using the size-adjusted MIC data (Bengtsson-Palme and Larsson 2016) and $PNEC_{sw}$ calculated from the

lowest NOEC in our meta analysis with the $PNEC_{fw}$ (PNEC in freshwater) determined for the 5 antibiotics in the European commission environmental quality standards watch list (Carvalho et al. 2015). In each case the $PNEC_r$ represents the highest PNEC for each antibiotic (i.e. is least protective as a whole).

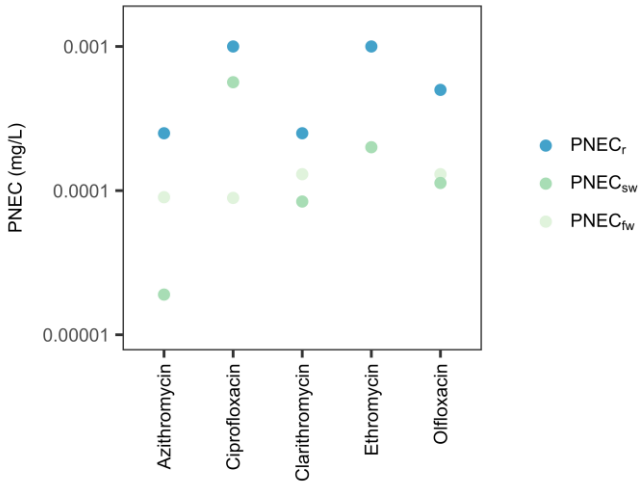

Fig 1. Predicted no effect concentrations (PNEC) for the antibiotics in the European commission watch list under the environmental quality standards directive (Carvalho et al. 2015). $PNEC_{fw}$ is the PNEC that is determined for freshwater in the European commission directive (Note that the assessment factor for $PNEC_{fw}$ may be up to 50 rather than 10 in these examples due to the lack of a full phase II base set of data – algae/cyanobacteria, invertebrates and fish (EMA 2006). The $PNEC_{fw}$ for ciprofloxacin is thus most likely overprotective); $PNEC_r$ is the PNEC calculated from minimum inhibitory concentrations (Bengtsson-Palme and Larsson 2016); $PNEC_{sw}$ is the PNEC determined from the lowest, publically available, environmental bacteria no observed effect concentration (Le Page et al. 2017). $PNEC_{sw}$ uses an assessment factor of 10 for each antibiotic.

As Bengtsson-Palme and Larsson (2018) point out, protection against antibiotic pollution for environmental health is more of a localised impact, whereas AMR has a wider and more pervasive global significance, directing stakeholders towards the need for two different protection targets determined from appropriate data and methodologies. We still maintain however, that an overall protection limit should protect both environmental and human health. Environmental protection and associated legislation differs across countries, but equally there is a social responsibility to ensure that product provenance is conducted to the highest possible levels.

Discharge limit

In response to stakeholder calls to address the risk of antibiotics released from manufacturing operations, which currently sits outside of the regulatory ERA framework, in our original paper we proposed an interim production discharge limit of 100 ng/L for each antibiotic, to be applied in the mixing zone to both protect environmental bacteria populations and reduce the risk of AMR development. This interim limit recognised that (i) because most antibiotics were authorised before the current guidelines came into force, many either lack or have very limited ecotoxicology data, and (ii) the need to establish science-based limits in the absence of such data. We were explicit in our paper to point out, however, that as sufficient data become available for mode of action relevant species we support the use of higher or lower protection limits based on these empirical data. Bengtsson-Palme and Larsson (2018) questioned this conservative limit for antibiotics because it may incur higher manufacturing costs through the need for infrastructure investment to reduce discharges and based on the fact that some antibiotics have relatively low toxicity and do not exert a strong selection pressure for antibiotic resistance. These are important points to debate. A single interim value helps the pharmaceutical industry, many of whom are currently reviewing their antibiotic manufacturing operations, to prioritise interventions and actions. These interventions may include generating relevant environmental toxicology data where empirical data does not exist or when a possible risk is identified at a site. A single value will also enable the pharmaceutical industry to benchmark existing suppliers more effectively to identify best practice in waste management. The requirement for infrastructure investments, as highlighted by Bengtsson-Palme and Larsson (2018), represents a last resort and these would only be required where risks could not be refined and managed through other interventions. Where infrastructure upgrades are required to meet scientifically robust limits, then the costs of these upgrades will need to be evaluated and justified as part of a wider socio-economic assessment into the stewardship of antimicrobial chemotherapy. In most cases, however, these interventions are not likely to incur excessive costs; the manual wipe down of equipment prior to cleaning washes, separation and incineration of the wastewater from the first wash of equipment, or the installation of inline filters

to remove undissolved material can all significantly reduce environmental concentrations of APIs, in most cases by >90% (Hargreaves et al. 2017). The logistics for antibiotic supply can be extremely complex with many suppliers manufacturing a whole range of antibiotics for numerous contractors and there can be language barriers and many suppliers lack the expertise to determine safe concentrations for themselves. In this case the use of a single interim limit has practical as well as scientific value. It may help remove conflicting limits (e.g. where two contractors provide different safe values or no level of protection), and minimise confusion amongst the pharmaceutical industry and their suppliers in the absence of data.

References

Antwis, R.E.; Griffiths, S.M.; Harrison, X.A.; Aranega-Bou, P.; Arce, A.; Bettridge, A.S.; Brailsford, F.L.; de Menezes, A.; Devaynes, A.; Forbes, K.M.; Fry, E.L.; Goodhead, I.; Haskell, E.; Heys, C.; James, C.; Johnston, S.R.; Lewis, G.R.; Lewis, Z.; Macey, M.C.; McCarthy, A.; McDonald, J.E.; Mejia-Florez, N.L.; O'Brien, D.; Orland, C.; Pautasso, M.; Reid, W.D.K.; Robinson, H.A.; Wilson, K.; Sutherland, W.J. Fifty important research questions in microbial ecology. FEMS Microbiology Ecology 2017;93:fix044-fix044

Bengtsson-Palme, J.; Larsson, D.G.J. Concentrations of antibiotics predicted to select for resistant bacteria: Proposed limits for environmental regulation. Environment International 2016;86:140-149

Bengtsson-Palme, J.; Larsson, D.G.J. Protection goals must guide risk assessment for antibiotics. Environment International 2018;111:352-353

Carvalho, R.; Ceriani, L.; Ippolito, A.; Lettieri, T. Development of the First Watch List Under the Environmental Quality Standards Directive. Directive 2008/105/EC, as Amended by Directive 2013/39/EU, in the Field of Water Policy. Joint Technical Report EUR 27142 EN; 2015

Delgado-Baquerizo, M.; Giaramida, L.; Reich, P.B.; Khachane, A.N.; Hamonts, K.; Edwards, C.; Lawton, L.A.; Singh, B.K. Lack of functional redundancy in the relationship between microbial diversity and ecosystem functioning. Journal of Ecology 2016;104:936-946

EC. Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use. in: European Union, ed. Brussels, Belgium; 2001

EMA. GUIDELINE ON THE ENVIRONMENTAL RISK ASSESSMENT OF MEDICINAL PRODUCTS FOR HUMAN USE. CPMP/SWP/4447/00 Corr 2. 2006

Hargreaves, C.; Hutchinson, K.; Snape, J.; Waern, F. Something in the water? The Chemical Engineer; 2017

Le Page, G.; Gunnarsson, L.; Snape, J.; Tyler, C.R. Integrating human and environmental health in antibiotic risk assessment: A critical analysis of protection goals, species sensitivity and antimicrobial resistance. Environment International 2017;